

PRODUCTION OF AN ANTIRONIDASE HORSE SERUM
AND ITS ACTION ON METASTASIZATION OF
A BROWN-PEARCE CARDINOMA IN RABBITS

I. N. Maiskii, N. A. Kozlova and M. N. Nilovskii

From the Laboratory of Noninfectious Immunology (Head — Prof. I. N. Maiskii)

Institute of Experimental Biology (Director — Prof. I. N. Maiskii)

AMN SSSR, Moscow

(Presented by Active Member AMN SSSR N. N. Zhukov-Verezhnikov)

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In previous work we showed depression of metastasization of a Brown-Pearce carcinoma in rabbits, after removal of the primary tumors, under the influence of an antironidase goat serum. Since the subject of the investigation is of practical as well as theoretical interest, an extension of the work in this direction is extremely important.

The object of the present investigation was to ascertain whether an antironidase serum, obtained by immunization of horses, possesses an inhibiting action on metastasization of a Brown-Pearce carcinoma.

METHOD

As donors of antironidase sera we used two horses (mare No. 11 and stallion No. 13). Immunization* of the animals was done in two cycles. The first cycle consisted of seven intramuscular injections of ronidase in doses of 1.5 to 8 g per injection. As a result of the first cycle of immunization, however, we were unable to induce a sufficiently high level of antibody production. The highest titer of antironidase antibodies in the McClean-Smirnova reaction was 1 : 64.

The subsequent immunization of the horses was temporarily postponed on account of the development of areas of infiltration at the sites of injection of the enzyme into the animals.

Six months later the horses were reimmunized by means of four intramuscular injections of 6, 15, 20 and 22.5 g ronidase, with intervals of 5-8 days between injections. During both immunization and reimmunization observations were made on the trend of antibody formation. At the moment of appearance of antibodies in a sufficiently high titer (on the ninth day after the last injection of ronidase) bleeding was carried out and the sera prepared.

The sera obtained were used for experiments after 2.5 months. The serum of horse No. 13, tested at this period by the McClean reaction, suppressed the activity of the homologous enzyme ronidase in a dilution of 1 : 4096, testicular enzyme of the rabbit and guinea pig in a dilution of 1 : 256, and testicular enzyme of the rat in a dilution of 1 : 64. The serum of horse No. 11 depressed the activity of these enzymes in the following dilutions respectively: ronidase 1 : 1024, testicular enzyme of the rabbit and rat 1 : 128 and guinea pig enzyme 1 : 256.

* Immunization was carried out at the Gamaleya Institute under the supervision of A. V. Ushakova, to whom we express our gratitude.

TABLE 1

Degree of Metastasization of a Brown-Pearce Carcinoma in Rabbits after Removal of the Tumor and Injection of Horse Sera (Sacrificed on the 21st Day after Inoculation of the Tumor)

Group of rabbits	Tumor	Serum	No. of rabbits	Mean number of affected organs per rabbit	Mean number of metastase* per affected organ	Weight of omentum (in g)	Weight of spermatic cord (in g)
First	Removed	Antironidase	4	3.2	8.3	15.	6.0
Second	"	Normal	4	5.7	20.1	25.7	6.0
Third	"	No serum injected	4	3.0	25.1	37	10.1
Fourth	Not removed	The same	4	4.7	44.6	24.2	

* In counting the number of metastases per affected organ, the metastases in the spermatic cord and omentum were disregarded.

Both sera were used in the experiments, which were conducted as follows: 39 male chinchilla rabbits, weighing 2.5-3.2 kg, were given an injection of 1 ml of a 25% suspension of a Brown-Pearce tumor into the left testicle. All the animals were then subsequently divided into three groups of equal weight. On the sixth day of development of the tumor, the rabbits of the first group received an intravenous injection of 6 ml of antironidase horse serum. The rabbits of the second group received an injection of the same volume of normal horse serum. The animals of the third group received no serum whatever.

On the seventh day the left testicle of 35 rabbits was removed and examined for the presence of a tumor. In four rabbits of the third group (Nos. 5, 90, 65 and 36) the testicle was not removed, and these animals then formed the fourth group.

On the next day, the eighth after transplantation of the tumor, 5 ml of the corresponding serum was injected intratesticularly into the rabbits of the first and second groups. Subsequently, at intervals of 2 days, each rabbit received a further four injections, each of 6 ml, alternately into the right and left posterior muscles of the thigh. Thus, altogether 6 injections were given, 2 intravenously and 4 intramuscularly.

On the 21st day after inoculation of the tumor, 16 rabbits, i.e., 4 from each group, were sacrificed. The remaining rabbits were sacrificed on the 36th day.

The effectiveness of the action of the sera on metastasization was estimated by the degree to which the organs were affected by metastases. As an index of the latter we used the number of organs affected by metastases, the average number of metastases in one affected organ and the weight of the omentum and the spermatic cord. The technique of counting the metastases in the organs was described in detail in the previous paper [1].

The results obtained at sacrifice of the animals on the 36th day were treated statistically by the Fisher-Student method. The differences were considered significant if the value of P was not greater than 0.05.

RESULTS

As the figures in Table 1 show, on the 21st day after transplantation of the tumor, in the rabbits from which the primary focus had been removed and which had received injections of normal horse serum (second group) and also in the rabbits which had received no treatment whatever (fourth group) the average number of affected organs was 5.7 and 4.7 respectively.

In the rabbits receiving antironidase serum (first group) the value of this index at this time was lower (3.2), although it did not differ from that in the group of animals not receiving serum after removal of the tumor (third group), for which it was (3.0).

Comparison of the mean indices of the number of metastases per affected organ shows that the density of metastasis formation in the organs of the experimental group (first group) was the lowest (8.3) in relation to all

TABLE 2

Degree of Metastasization of a Brown-Pearce Carcinoma in Rabbits after Removal of the Tumor and Injection of Horse Sera (Sacrificed on the 36th Day after Inoculation of the Tumor)

Group of rabbits	Tumor	Serum	Rabbit No.	Number of affected organs	Mean No.* of meta-stases per affected organ	Omentum		Spermatic cord	
						degree of involvement	wt. (in g)	degree of involvement	wt. (in g)
First	Re-moved	Anti-ironidase	9	—	—	—	24,0	—	1,5
			12	1	—	++	8,0	—	1,0
			8	2	2,0	—	11,0	+	3,0
			54	5	4,3	+	34,5	+	11,0
			42	7	4,6	+	19,0	+	5,0
			30	8	25,6	+	12,5	++	55,0
			7	9	11,4	++	40,0	+	7,0
			2	9	15,0	++	74,5	++	26,5
Mean				5,1	7,9		27,9		13,7
Second	Re-moved	Normal	16	1	1,0	—	13,0	—	2,0
			24	1	1,0	—	8,0	—	2,0
			23	4	12,5	+	15,0	+	60,0
			6	6	26,6	+	41,0	++	45,5
			52	7	30,2	++	74,0	++	67,0
			81	8	34,8	+++	141,0	++	35,0
			70	10	47,4	+++	225,0	++	76,0
			75	11	84,5	+++	190,0	++	28,0
			57	11	162,3	+++	162,0	++	55,0
Mean				6,5	44,5		96,55		41,2
Third	Re-moved	No serum injected	61	7	20,2	+	12,0	+	5,5
			1	8	46,8	++	65,0	++	47,0
			83	8	94,4	++	50,0	++	60,0
			55	11	48,5	+++	150,0	++	60,0
			87	9	153,0	+++	185,0	++	36,0
Mean				8,6	72,6		92,4		41,7

Conventional signs: — metastases absent; + few metastases (from 1 to 10); ++ multiple metastases (from 11 to 50); +++ metastases could not be counted (large, confluent nodules of metastases).

*In counting the mean number of metastases per affected organ, the metastases in the spermatic cord and omentum were disregarded.

the control group (including the third group), in which the average number of metastases was 20.1 (in the second group), 25.1 (in the third group) and 44.6 (in the fourth group).

Involvement of the omentum was also least pronounced in the animals of the first (experimental) group (15 g against 25.7, 37.0 and 24.2 g in the controls).

The development of the tumor in the spermatic cord was more marked in the third group (average weight 10.1 g) than in the first and second groups (6.0 g).

Since in the fourth group (technical control) the testicle with the tumor was not removed, no comparison of the degree of involvement of the spermatic cord was made. It should be pointed out, however, that in all the rabbits of this group the tumor developed and reached considerable dimensions (weight of the testicle with the tumor in rabbits No. 5 — 24 g, No. 36 — 18 g, Nos. 90 and 65 — 12 g). This indicates that the strain of Brown-Pearce tumor which we used possessed high virulence.

In Table 2 we give the results showing the degree of metastasization of the Brown-Pearce carcinoma in the rabbits at the time of their sacrifice on the 36th day. It should be pointed out that three rabbits died earlier than the stated period (rabbit No. 57 on the 24th day, No. 87, on the 25th day and No. 70 on the 35th day). Since these rabbits died as a result of intensive metastasization of the tumor, we considered that they could be included in this table. One rabbit (No. 56) died from an accidental cause and was not included in the experiment.

The experimental results also showed that the highest number of affected organs (8.6 of the 12 organs examined) was observed in the third group of animals, which received no serum, and the lowest was found in the first group — in the rabbits receiving antironidase serum (5.1 organs). In the rabbits of the second group, which received injections of normal horse serum, the average number of affected organs was 6.5.

The smallest number of metastases per affected organ was observed in the rabbits of the experimental group — 7.9, whereas in the second group, after injection of normal horse serum, the number of metastases was 47.5 and in the third group, in which the rabbits received no serum, it was 72.6. The statistical differences between the results for the first and second, and the first and third groups are significant (in the first case $P = 0.053$, in the second $P = 0.005$). Meanwhile, the injection of normal serum led to no significant decrease in the number of metastases. The difference between the mean values for the second and third groups is not significant ($P = 0.384$). This demonstrates that the degree of involvement of the organs with metastases was least after injection of antironidase serum.

The same relationship was observed when a comparison was made of the weight of the omentum and of the spermatic cord in the experimental and control animals (since the weight of the omentum and of the spermatic cord depends on the degree of development of metastases of the tumor therein). The weight of the omentum in the first group (experimental) was 27.9 g, in the second group 96.5 g and in the third group 92.4 g. The probability of a chance difference between the indices of the first and second and of the first and third groups is significant (in the first case $P = 0.036$ and in the second $P = 0.030$), but that between the indices of the second and third groups is not significant ($P = 1$). The weight of the testicle in the rabbits of the first group was 13.7 g, in the second group 41.2 g and in the third group 41.7 g. The difference between the indices in the first and second groups is significant ($P = 0.024$), whereas that between the indices of the second and third control groups is not significant ($P = 1.0$).

The injection of antironidase horse serum thus affords considerable protection against the development of metastases. This was reflected in the decrease in the number of metastases in the organs of the animals receiving antironidase serum by roughly 5.6 times compared with animals receiving injections of normal horse serum and by 9.2 times compared with the animals receiving no treatment. Moreover, in the animals receiving antironidase serum, metastasis formation in the omentum and spermatic cord was minimal. A slight decrease in the incidence of involvement of the organs by metastases was also observed.

SUMMARY

Antironidase serum was obtained by immunizing 2 horses with ronidase. This serum was tested on rabbits after removal of a Brown-Pearce tumor. Metastasization was depressed by this serum: there was a reduction of the mean number of metastases in the affected organs, of the degree of metastasization in the omentum and spermatic cord, as well as of the number of organs affected by metastases.

LITERATURE CITED

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*Original Russian pagination. See C. B. translation.